# BIOASSAY AND EFFICACY OF *BACILLUS THURINGIENSIS* AND AN ORGANOSILICONE SURFACTANT AGAINST THE CITRUS LEAFMINER (LEPIDOPTERA: PHYLLOCNISTIDAE)

Jeffrey P. Shapiro,[1,3] William J. Schroeder[1] and Philip A. Stansly[2]
[1]USDA, Agricultural Research Service, 2120 Camden Road, Orlando, Florida 32803

[2]University of Florida - IFAS, SWFREC, P.O. Drawer 5127, Immokalee, FL 33934

[3]Corresponding author; email: jshapiro@asrr.arsusda.gov

## Abstract

Two laboratory bioassays were developed for testing insect pathogens for control of larval citrus leafminer, *Phyllocnistis citrella* Stainton, in isolated citrus leaves. An organosilicone surfactant and several commercial preparations of *Bacillus thuringiensis* (Berliner) (*Bt*) were tested in these bioassays. One bioassay tested the effect of direct contact of a test mixture on second or third instars by injecting the mixture into a leaf mine surrounding the head of a larva. The second bioassay tested the effect of a test mixture when topically applied to the surface of a leaf mine above a larva. Effects in this second bioassay were only observed if the mixture or its components first penetrated through the mine epidermis, and then contacted and affected the insect within the mine. In both bioassays, blue food coloring was included as a tracer to observe movement of liquid into mines and through the gut of the larva. An organosilicone surfactant, Silwet L-77, enhanced penetration of solutions into mines and killed larvae when applied topically at adequate concentrations ($LC_{50}$ = 0.026% in water) or when injected into mines ($LC_{50}$ = 0.011%). Three commercial *Bt* preparations were active when applied topically at high concentrations or when injected at lower concentrations. When L-77 was added at its $LC_{10}$ (0.01%) to *Bt* in the topical bioassay, activity of one of the three *Bt* preparations, or activity of all three preparations considered together, was significantly higher than without the L-77. Up to 90% mortality resulted at 0.01% L-77 and 20% *Bt*. Mortality positively correlated with dose of all of the *Bt*s when topically applied with 0.01% L-77. These results suggested that an increased concentration of surfactant and decreased concentration of *Bt* at commercial rates might be effective against citrus leafminers in citrus groves or nurseries. This was tested on old nursery trees (3 yr after planting) and young grove trees (2 yr after planting). Application of any of the three *Bt*s plus L-77 in the nursery resulted in reduced populations of live larvae and pupae in new foliage, to approximately 50% of the population in water-treated controls. *Bt*s without L-77 yielded counts that were intermediate to, but not significantly different from, control and combined *Bt*-L-77 treatments. *Bt* applied with L-77 in the grove did not significantly reduce percentage damage to leaves and number of larvae per leaf when assessed at 14 days. However, when assessed at 21 days, damage and number of larvae were significantly reduced by treatment with *Bt* plus L-77 or L-77 alone. *Bt* and L-77 reduced leaf damage and larval numbers to a level not significantly different from treatment with Agri-Mek and spray oil. Assessed at 14 or 21 days, the L-77 treatment resulted in significant reduction of larvae.

Key Words: *Phyllocnistis citrella*, citrus leaf penetration, bioassay, citrus nursery, citrus grove

## Resumen

Se diseñaron dos bioensayos para experimentar con el uso de entomopatógenos para el control de larvas del minador de los cítricos, *Phyllocnistis citrella* Stainton, en hojas de cítrico aisladas. Un surfactante organo-silíceo, varias preparaciones comerciales de *Bacillus thuringiensis* (Berliner) (*Bt*), y mezclas de ellos fueron ensayados. En un ensayo se evaluó el efecto del contacto directo de una mezcla de *Bt* con surfactante en larvas del segundo o tercer estadío, inyectando la mezcla de prueba dentro de las minas de las hojas rodeando la cabeza de las larvas. En el segundo ensayo se determino el efecto de una mezcla de prueba aplicada directamente en la superficie de la mina de la hoja, arriba de la larva. En este ensayo sólo se observó efecto alguno si la mezcla o sus componentes penetraban la epidermis de la mina y entraban en contacto con el insecto dentro de la mina. En ambos bioensayos, un colorante azul de alimentos fue añadido como trazador para observar el movimiento del líquido dentro de las minas y a través del sistema digestivo de la larva. El surfactante organo-silíceo, Silwet L-77, incrementó la penetración de las soluciones dentro de las minas y mató larvas cuando se les aplicó tópicamente en una concentración adecuada ($LC_{50}$ = 0.026% en agua) o cuando se inyectó dentro de las minas ($LC_{50}$ = 0.011%). Tres preparaciones comerciales de *Bt* resultaron ser activas cuando se aplicaron tópicamente en altas concentraciones o cuando se inyectaron en bajas concentraciones. Cuando se aplicaron tópicamente mezclas de L-77 al $LC_{10}$ (0.01%) con *Bt* se observó un aumento en la actividad de *Bt* en comparación con la aplicación de *Bt* sin L-77. Se obtuvo hasta un 90% de mortandad cuando se utilizó L-77 al 0.01% y *Bt* al 20%. La mortandad resulto estar correlacionada positivamente con todas las dosis de *Bt* aplicadas tópicamente junto con L-77 al 0.01%. Estos resultados sugirieron que se podría reducir la concentración de *Bt*, aumentando la concentración del surfactante, y aún mantener su efectividad contra los minadores en plantaciones o en viveros de cítricos. Esta hipótesis se probó en un vivero con árboles viejos (de 3 años después de ser sembrados) y en una plantación con árboles jóvenes (de 2 años después de ser sembrados). La aplicación en el vivero de los tratamientos con cualquiera de tres dosis diferentes de *Bt* con L-77 ocasionó una reducción de aproximadamente un 50% de la población de larvas y pupas vivas en el follaje nuevo en comparación con controles tratatados con agua. Tratamientos con *Bt* sin L-77 dieron lugar a conteos que fueron intermedios, pero no significativamente diferentes de resultados del control y de los tratamientos con *Bt* y

L-77. Tratamientos con *Bt* y L-77 ensayados en plantaciones no redujeron significativamente el porcentage de daño en las hojas y el número de larvas por hoja cuando evaluados a los 14 días. Sin embargo, en evaluaciones hechas 21 días después del tratamiento, se encontró que tanto el daño como el número de larvas se redujeron significativamente en tratamientos con *Bt* y L-77 y también con L-77 solamente. Tratamiento con *Bt* y L-77 redujeron el porcentaje de daño en las hojas y el número de larvas de *Phyllocnistis citrella* a un nivel no significativamente diferente al observado con un tratamiento de Agri-Mek y aceite. Evaluaciones hechas a los 14 o 21 días después de la aplicación de L-77 demostraron una reducción significativa en el número de larvas.

The citrus leafminer, *Phyllocnistus citrella* Stainton, was first detected in Florida in May of 1993 (Heppner 1993). Within six months, the tiny moth had spread throughout the state. Based upon experience in China, Thailand, and Australia, biological control with parasitoids offered the best chance for effective long term management (Beattie & Smith 1993). However, effective short term controls that are not disruptive to natural enemies are required when leafminer populations are too high to be quickly suppressed by parasitoids, or prior to establishment of parasitoids.

The epidermis of the citrus leaf provides substantial protection for leafminers and presents a significant barrier to control using chemical or microbial insecticides. To enter the mine and contact a larval leafminer, a microbial or chemical agent must first penetrate the epidermal cell layer, either through stomata in the abaxial leaf surface or between or through epidermal cells. The extremely low surface tension of organosilicone surfactants such as Silwet L-77 (L-77) in aqueous solution (Knoche et al. 1991) may greatly increase penetration of a chemical insecticide (Stevens 1993), herbicide (Reddy & Singh 1992a, 1992b; Singh & Mack 1993) or a microbial agent (Zidack et al. 1992). In addition, these compounds may offer some protection themselves, as seen against the green peach aphid, *Myzus persicae* (Sulzer) (Imai et al. 1994).

In this paper we describe and compare results from two bioassays applied against leafminers in isolated citrus leaves. A mine injection bioassay tested the lethalities of L-77 and/or *Bt* when they were directly applied to leafminers, circumventing the epidermal barrier. A topical bioassay tested the penetrability and resulting lethality of L-77 and *Bt* when they were applied to the surface of intact leaf mines, with the epidermal barrier intact. These tests demonstrated an enhanced effect of *Bt* applied with L-77, and the effect was further tested in field trials in a citrus grove and a nursery.

## Materials and Methods

### Chemicals & *Bt* Preparations

Silwet L-77 (OSI, Tarrytown, NY) and commercial preparations of *Bt* (Condor, Ecogen, Langhorne, PA; Florbac HPWP and Biobit HPWP, Entotech Company, Davis, CA) were supplied by manufacturers. Biobit is a strain of HD-1 (*Bt kurstaki*, with genes coding for *Cry*1 and *Cry*2 toxins). Florbac is a *Bt* aizawa strain HD-11 (with a gene coding for *Cry1*C). Condor is a proprietary transconjugate. Water used in laboratory studies was purified by deionization, reverse osmosis, and finally through a water purification system (Millipore MilliQ, Bedford, MA). Agri-Mek (Merck & Co., Rahway NJ), a formulation of abamectin which provides good chemical control against leafminers, was used as a positive control in the grove trial.

Laboratory Bioassays

Two bioassays were devised to test toxicities of an organosilicone surfactant, Silwet L-77, and various commercial *Bt* formulations against citrus leafminers in intact leaves isolated from trees. A mine injection assay directly and immediately subjected larvae to test mixtures within leaf mines. A topical bioassay directly exposed only the surface of the mines to test mixtures. Any exposure of larvae in the topical bioassay occurred only after the test mixture or its ingredients penetrated through the intact mine epidermis to contact a larva within the mine.

Solutions and suspensions were mixed in 1.5-ml microcentrifuge tubes with blue food coloring (FD&C Blue dye No. 1, McCormick Company) added to 10% of final volume to allow microscopic observation of penetration through the mine epidermis, ingestion by leafminers, and movement through their guts. The same dye was included in all checks. A flowable *Bt* formulation (Condor) was mixed by volume, wettable *Bt* powders (Biobit or Florbac) were mixed by weight, and Silwet L-77 was mixed by volume after serial dilution in water. Solutions were then brought to final volumes with water and mixed with a vortex mixer prior to application.

Leaves containing leafminer larvae were excised complete with petioles from grapefruit seedlings ('Marsh' and 'Inman' cultivars) that were grown in 5-gal pots outdoors where newly developing leaves (flush) were infested by leafminers. Only leaves that contained actively feeding second or third instars were used for bioassays.

In the mine injection bioassay, 4 µl of test solution were applied to the surface of a mine in a droplet above the head of a leafminer under a stereomicroscope. The test solution was then introduced into the mine by puncturing the mine epidermis with a No. 0.10 minutin pin to allow influx of the solution into the mine surrounding the head of the larva. For the topical bioassay, 4 µl of solution were placed onto the mine surface directly above the head of an actively feeding larva without puncturing the mine.

Following injection or topical treatment of mines, the tip of the petiole was trimmed from each leaf and the leaf was placed into a 2-ml water-filled vial, with its petiole retained by a wet cotton wick. Vials were maintained in the laboratory at room temperature (about 23°C) inside a closed plastic box (19 × 13.5 × 10.5-cm). Mortality was recorded when larvae exhibited a total lack of external or peristaltic movement when probed, and was assessed daily. Statistics were calculated by personal computer using POLO-PC (LeOra Software, Berkeley, CA) (Russell & Robertson 1979) for probit analysis and Statistica (StatSoft, Tulsa, OK) for other statistics.

Nursery Trial

To translate and compare laboratory results to a field situation, a citrus nursery trial was conducted near Lake Jem in northern Orange County, Florida on a single row of a 2-year old planting of 'Marsh' grapefruit trees on citrumelo rootstock. Eight treatments were assigned in a randomized block design with 5 blocks, each including three trees per treatment. Trees were hedged, fertilized 2 days later, and allowed to flush and become infested with leafminer larvae. Treatments were applied 16 days after hedging. *Bt* preparations were mixed by volume at 0.32% of stock commercial preparation in water, L-77 at 0.05%. Five days following treatment, 1-3 flushes appeared on each young tree. Each flush consisted of a cluster of young developing leaves, averaging 12 leaves/tree, with a mean leaf surface area of 201 ± 58 $cm^2$/flush. One flush from the top of each tree was randomly selected and collected, and numbers of live and dead larvae and pupae were counted in the laboratory under a stereomicroscope and recorded. Combined counts of live larvae plus live pupae are reported. Statistics were calculated by personal computer using Statistica.

Grove Trial

To determine whether treatment with a *Bt* and L-77 could effect control that approached the control achieved by an effective insecticide treatment, Biobit and L-77 were tested against Agri-Mek + 435-66 spray oil in a citrus grove trial. The trial was performed near Immokalee, FL on a two-year-old block of 'Valencia' orange budded to 'Swingle' citrumelo rootstock. Ten treatments were assigned to 5-tree plots in a completely randomized block design, each block as a separate row, with 4 replications. Treatments were applied during one day by a handheld atomizing spray gun supplied by a gasoline-powered diaphragm pump, calibrated to deliver 702 l/ha (75 gal/ac) at 2.8 MPa (400 psi). Ten samples of flush per plot were examined in the field. Live larvae per leaf were counted and damage was visually estimated as percentage of total leaf surface. Statistics were calculated by personal computer using SAS (SAS Institute, Cary, NC).

## RESULTS

At 50-fold magnification, the roof of the mine above active leafminers was seen to be composed of intact leaf epidermis. Larvae fed on parenchymal tissue below the epidermis with coordinated sweeping semicircular movements of the mandibles. Parenchymal tissue, macerated and liquefied by the mandibles, rapidly entered the pharynx and foregut and continued through the digestive system. When mines were injected with a solution containing only 10% blue food coloring in water, movements of head and mandibles ceased within 10 s, but peristalsis of pharynx and gut continued until liquid was fully ingested and removed from the mine chamber around the head. Mandibular and head movements and ingestion of parenchymal tissue resumed once liquid had been cleared. Ingestion and excretion of dyed liquids were easily observed. Fifteen to 20 min elapsed between ingestion and excretion. Ninety to 100% of larvae in the control (water-fed, including 10% blue food coloring) treatments survived to pupation.

Silwet L-77 killed larvae when bioassayed by mine injection or topical treatment, and mortality increased with concentration (Fig. 1). Leafminers were 1.5- to 3-fold more susceptible to mine injection than to topical treatment of mines with L-77, as demonstrated by using probit analysis to calculate the injected concentrations lethal to 10%, 50%, and 90% of larvae ($LC_{10}$, $LC_{50}$, and $LC_{90}$, respectively) (Table 1). The time required for L-77 to kill larvae was also dependent on concentration. With topical application of 0.5% L-77, feeding ceased after 10 min and peristalsis ceased after 16 min; at 0.1%, cessation of functions took 3 h. Whether mines were injected or topically treated, larvae did not recover once gut peristalsis ceased.

In initial tests of Condor *Bt* applied to leaf mines in isolated leaves, a 20% solution with 10% blue food coloring in water was injected into the mines of five larvae. Within 1-2 h of treatment, four of five larvae stopped feeding, midgut peristalsis halted, and they died within 22 h. Next, suspensions of three commercial *Bt* preparations, two wettable powders (Biobit and Florbac) and a flowable concentrate (Condor), were topically applied to leaf mines in solutions with or without L-77 (Table 2). L-77 was included at a concentration (0.01%) equivalent to the $LC_{10}$ (Table 1). This concentration was calculated to cause minimal mortality yet still enhance penetration of food coloring, judged by visual observation.

Leaf miners in isolated leaves that were topically treated with one of the three *Bt* preparations or with L-77 + *Bt* sustained significantly higher mortalities than those treated with L-77 only (Table 2). Treatment with L-77 + *Bt* resulted in significantly higher mortality than *Bt* alone when Biobit was used, or when the grand means from

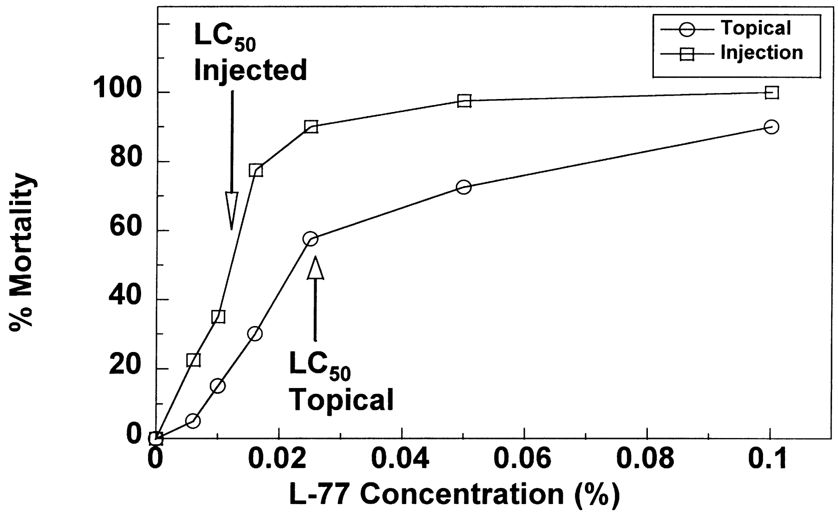


FIG. 1. Mortality of citrus leafminer larvae in isolated leaves with increasing concentrations of L-77 in water applied topically to leaf mines or injected into them. L-77 was serially diluted to the given concentrations in water containing 10% blue food coloring, then applied in the topical or mine injection bioassays.

all three *Bt* preparations were compared (10.0% mean mortality in combined L-77 treatments, 64.1% with *Bt*, and 82.5% with L-77 + *Bt*). Condor and Florbac treatments showed the same progression, but with no significant difference (by Tukey's HSD test) between *Bt* and L-77 + *Bt* treatments.

Mortality of leafminers in isolated leaves increased with *Bt* concentration when mines were topically treated with *Bt* in 0.01% L-77 (Table 3). $LC_{50}$ values (lower and upper 90% confidence limits) of Biobit, Condor, and Florbac were calculated by probit analysis as 0.29% (0.13, 0.63), 0.14% (0.043, 0.36), and 1.74% (0.45, 41.3) concentrations, respectively (v/v of Condor or w/v of Biobit and Florbac). Goodness of fit of the probit model was significant ($g < 0.5$) at the 99% confidence level for Biobit and Condor, but only at the 90% confidence level for Florbac.

Field trials with *Bt* preparations and L-77 were run in a nursery and a grove. In the dense foliage of a citrus nursery, populations of the citrus leafminer built very rap-

TABLE 1. LETHAL CONCENTRATIONS AND 95% CONFIDENCE LIMITS FROM PROBIT ANALYSIS OF SILWET® L-77 APPLIED TOPICALLY OR INJECTED INTO LEAF MINES.

| | Topical | | Injected | |
|---|---|---|---|---|
| Lethal concentration | Dose (%) | 95% Conf. Limits | Dose (%) | 95% Conf. Limits |
| $LC_{10}$ | 0.007 | 0.005-0.010 | 0.005 | 0.003-0.006 |
| $LC_{50}$ | 0.026 | 0.022-0.032 | 0.011 | 0.009-0.013 |
| $LC_{90}$ | 0.091 | 0.067-0.140 | 0.026 | 0.022-0.035 |

TABLE 2. PERCENTAGE MORTALITY OF LARVAE IN LEAF MINES TOPICALLY TREATED WITH WATER, SILWET L-77, ONE OF THREE *BT* FORMULATIONS, OR SILWET L-77 + *BT*.

| | Percentage Mortality[2] | | |
|---|---|---|---|
| *Bt* Preparation[1] | L-77 Control[3] | Bt | L-77 + *Bt* |
| Biobit HPWP | 7.5 ± 5.0 a | 62.5 ± 17.1 b | 87.5 ± 12.6 c |
| Condor | 12.5 ± 5.0 a | 79.7 ± 19.8 b | 90.0 ± 8.2 b |
| Florbac HPWP | 10.0 ± 8.2 a | 50.0 ± 21.6 b | 70.0 ± 11.5 b |
| Total | 10.0 ± 6.0 a | 64.1 ± 21.8 b | 82.5 ± 13.6 c |

[1]*Bt* formulations were diluted to 20% commercial strength in water (w/v for Biobit or Florbac, v/v for Condor). L-77 was diluted to 0.01% in water or in the *Bt*-water mixture.

[2]Means ± SD of 4 replications, 10 larvae/replicate; means followed by the same letter within a row are not significantly different by Tukey's HSD test following a 1-way analysis of variance (ANOV).

[3]All $H_2O$-treated controls, including blue food coloring, survived and were not included in the 1-way ANOV.

idly following hedging and subsequent synchronous flushing of nursery trees. This assured adequate leafminer populations for testing. Combined larval and pupal counts at 5 days following treatment were significantly lower than water controls on flush treated with L-77 plus any of the *Bt* preparations (Table 4). Those counts were lower than counts observed on flush treated with *Bt* alone, yet not significantly so by Tukey's HSD test. Neither L-77 alone, nor any of the *Bt* preparations without L-77, had a significant effect on counts, compared to the control treatment.

In an immature grove, results from application of Biobit *Bt* with L-77 did not show significant reduction in damage after 14 days, although larval populations were significantly reduced by L-77 alone (Table 5). Agri-Mek + oil showed significant reduction of both damage and larvae at 14 days. At 21 days, reductions of damage and larval populations were significant in flush treated with L-77, Agri-Mek + oil, or Biobit *Bt* + L-77. Damage and larval counts with *Bt* + L-77 treatment did not differ significantly from treatment with Agri-Mek + oil.

TABLE 3. PERCENTAGE MORTALITY OF LARVAE IN MINES TOPICALLY TREATED WITH VARYING CONCENTRATIONS OF COMMERCIAL *BT* PREPARATIONS WITH 0.01% L-77.

| | *Bt* Concentration | | | | |
|---|---|---|---|---|---|
| *Bt* Preparation | 0%[1] | 0.01% | 0.10% | 1% | 5% |
| Biobit HPWP[2] | 0 | 25.0 ± 5.8 | 35.0 ± 19.1 | 60.0 ± 8.2 | 77.5 ± 12.6 |
| Condor | 0 | 12.5 ± 5.0 | 62.5 ± 22.2 | 77.5 ± 18.9 | 72.5 ± 9.6 |
| Florbac HPWP | 0 | 17.5 ± 12.6 | 35.0 ± 23.8 | 30.0 ± 14.1 | 67.5 ± 22.2 |

[1]Results are means ± SD from 4 replicates of 10 larvae per replicate.

[2]Percentage w/v for Biobit and Florbac wettable powders and percentage v/v for Condor, all in 0.01% L-77.

TABLE 4. LIVE LARVAE AND PUPAE PER FLUSH 5 DAYS FOLLOWING TREATMENT OF GRAPEFRUIT IN A NURSERY WITH MIXTURES OF *BT* (0.32%) AND/OR L-77 (0.05%).

| Rank | Treatment | Mean Counts/Flush[1] ± SD |
|---|---|---|
| 1 | Water | 19.4 ± 6.3 a |
| 2 | Condor | 14.3 ± 9.2 ab |
| 3 | Florbac | 14.1 ± 7.6 ab |
| 4 | L-77 | 14.0 ± 9.5 ab |
| 5 | Biobit | 13.9 ± 7.7 ab |
| 6 | Condor + L-77 | 10.1 ± 6.5 b |
| 7 | Florbac + L-77 | 10.1 ± 5.2 b |
| 8 | Biobit + L-77 | 8.5 ± 5.9 b |

[1]Means followed by the same letter are not significantly different ($P < 0.05$; Tukey's HSD test; $N = 15$).

## DISCUSSION

Two complementary *in situ* bioassays were developed and their utility was demonstrated. An injection bioassay was used to assess direct activities of several biocontrol agents against larval citrus leafminers. A topical bioassay tested the ability of adjuvants and active biocontrol agents to penetrate into leaf mines and demonstrate activity against leafminers. Using these bioassays, we demonstrated that several commercial *Bt* preparations were active against the leafminer, at least at high concentrations. Furthermore, *Bt* clearly penetrated into leaf mines to affect the miners, and penetration and activity were enhanced by an organosilicone surfactant, L-77.

In laboratory tests, Silwet L-77 diluted in water killed leafminers when applied at sufficient concentrations. Imai et al. (1994) showed that Silwet L-77 also killed aphids. A 0.1% concentration of L-77 in water killed 100% of the aphids, a concentration comparable to the $LC_{90}$'s observed against citrus leafminers (0.091% topical, 0.026% by injection).

Field trials permitted scaleup and translation of laboratory methods to the field. Field application differed from laboratory bioassays in several important respects. In the bioassays, mine penetration and leafminer mortality were observed after L-77 was topically applied with blue food coloring, and these effects increased with increasing concentrations of L-77. Because L-77 produced high mortality in the bioassays at moderate to high concentrations, laboratory bioassays to test for L-77 enhancement of *Bt* activity included a low L-77 concentration (0.01%) with high concentrations of *Bt*. For field application, L-77 was added at 0.05% concentration. This higher L-77 application rate should therefore require lower concentrations of *Bt*, and results in Tables 4 and 5 indicate that *Bt* did show activity at concentrations close to the recommended rates. A typical field rate for *Bt* application is approximately 1-2 kg/Ha (1-2 lbs per ac) at 935 l/Ha (100 gals per acre); 1.12 kg/Ha (1 lb/ac) translates to 0.12% *Bt* concentration in sprays. In lab bioassays, we showed activity over controls (mixed with a low 0.01% L-77 only) at *Bt* concentrations as low as 0.1%.

Surfactants have long served as effective adjuvants, increasing the uptake, retention, and persistence of conventional pesticides and other agrichemicals (Stickle 1992). Recently, L-77 has been shown to enhance efficacy through stomatal penetration of plant pathogens of weeds used as biological control agents (Zidack et al. 1992). Organosilicone surfactants are particularly effective in increasing the efficacy of in-

TABLE 5. DAMAGE (PERCENTAGE OF LEAF SURFACE DAMAGED) AND LIVE LARVAE PER LEAF RECORDED 14 AND 21 DAYS AFTER TREATMENT OF VALENCIA ORANGE TREES IN A GROVE.

| Treatment | 14 Days | | 21 Days | |
|---|---|---|---|---|
| | % Damage | Larvae/Leaf | % Damage | Larvae/Leaf |
| Untreated | 18.9 a[2] | 1.2 a | 46.7 a | 1.7 a |
| L-77 | 13.4 a | 0.9 b | 9.5 b | 0.8 b |
| Biobit + L-77 | 15.5 a | 1.1 ab | 5.5 b | 0.5 bc |
| Agri-Mek + 435-66 Oil | 3.1 b | 0 c | 4.7 b | 0.3 c |

[1]Rates of application (alone or in mix; % by vol.): 435-66 oil, 2%; L-77, 0.05%; Biobit, 2.2 kg/ha; Agri-Mek, 586 ml/ha.
[2]Means in the same column followed by the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

secticides (Adams et al. 1988), herbicides, growth regulators, and foliar nutrients (Stevens et al. 1992). The exceptional activity of the organosilicones is probably due to their unique physiochemical properties and high activities as wetting agents (Goddard & Padmanabhan 1992). The high activity of L-77 clearly enables penetration of the very hydrophobic stomatal environment by aqueous solutions (Zidack et al. 1992). Our results demonstrate enhanced activity of topically applied *Bt* when applied with a low concentration of L-77, probably due to increased penetration through mine stomata into the mines. Since leafminers preferentially mine the abaxial surface of leaves, enhanced stomatal infiltration is especially useful against them.

L-77 penetrated leaf mines at concentrations lower than those required to kill miners, as observed by penetration of blue food coloring. The higher concentrations of L-77 required to induce mortality are consistent with drowning as a mode of action. In order to test whether L-77 increased penetrability of *Bt*, we used a low 0.01% concentration of L-77 that was nontoxic to larvae, yet penetrated into leaf mines and was ingested. At higher concentrations, organosilicone surfactants may further enhance penetrability of *Bt* into leaf mines. In addition, these surfactants may enhance the retention and persistence of *Bt* toxins beneath the leaf epidermis.

## ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Dorothy Hoppe, Nancy Maynard, Hunter Smith, and Greg Fulcher. Our thanks to Omelio Sosa, José Chaparro, and Martha Ramirez for work on the Resumen. Funds for this project were made available from the Citrus Production Research Marketing Order by the Division of Marketing and Development, Florida Department of Agriculture and Consumer Services. Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

## REFERENCES CITED

ADAMS, A. J., J. S. FENLON, AND A. PALMER. 1988. Improving the biological efficacy of small droplets of permethrin by the addition of silicon-based surfactants. Ann. Appl. Biol. 112: 19-31.

BEATTIE, G. A. C., AND D. SMITH. 1993. Citrus leafminer. Agfacts H2.AE.4: 1-6.
GODDARD, E. D., AND K. P. A. Padmanabhan. 1992. A mechanistic study of the wetting, spreading, and solution properties of organosilicone surfactants, pp. 373-383 in C. L. Foy [ed.] Adjuvants for Agrichemicals. CRC Press, Boca Raton.
HEPPNER, J. B. 1993. Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae: Phyllocnistinae). Florida Division of Plant Industry Entomology Circular 359: 1-2.
IMAI, T., S. TSUCHIYA, K. MORITA, AND T. FUJIMORI. 1994. Surface tension-dependent surfactant toxicity on the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Appl. Entomol. Zool. 29: 389-393.
KNOCHE, M., H. TAMURA, AND M. J. BUKOVAC. 1991. Performance and stability of the organosilicone surfactant L-77: Effect of pH, concentration, and temperature. J. Agric. Food Chem. 39: 202-206.
REDDY, K.N., AND SINGH, M. 1992a. Organosilicone adjuvant effects on glyphosate efficacy and rainfastness. Weed Technology 6:361-365.
REDDY, K.N., AND SINGH, M. 1992b. Organosilicone adjuvants increased the efficacy of glyphosate for control of weeds in citrus (*Citrus* spp.). Hortscience 27: 1003-1005.
RUSSELL, R. M., AND J. L. ROBERTSON. 1979. Programming probit analysis. Bull. Entomol. Soc. America 25: 191.
SINGH, M., AND MACK, R.E. 1993. Effect of organosilicone-based adjuvants on herbicide efficacy. Pestic.Sci. 38: 219-225.
STEVENS, P. J. G. 1993. Organosilicone surfactants as adjuvants for agrochemicals. Pestic. Sci. 38: 103-122.
STEVENS, P. J. G., J. A. ZABKIEWICZ, J. H. BARRAN, K. R. KLITSCHER, AND F. EDE. 1992. Spray formulations with Silwet organosilicone surfactants, pp. 399-403 in C. L. Foy [ed.] Adjuvants for Agrichemicals. CRC Press, Boca Raton.
STICKLE, W. E. 1992. The importance of adjuvants to the agricultural chemical industry, pp. 247-249 in C. L. Foy [ed.] Adjuvants for Agrichemicals. CRC Press, Boca Raton.
ZIDACK, N. K., P. A. BACKMAN, AND J. J. SHAW. 1992. Promotion of bacterial infection of leaves by an organosilicone surfactant: implications for biological weed control. Biol. Control 2: 111-117.